NICEATM

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods

Nomination of *In Vitro* Assays for the Detection and Quantification of Botulinum Neurotoxins

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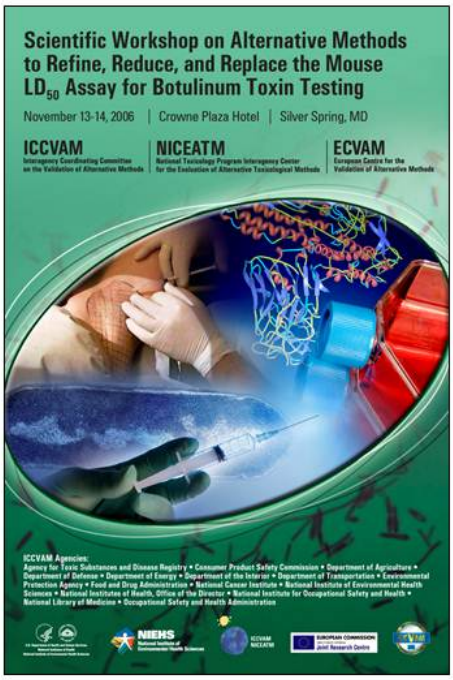
SACATM Meeting
June 16, 2011
Hilton Arlington,
Arlington, VA

Botulinum Neurotoxin (BoNT) Testing

- BoNT is the most toxic substance known
  - Proteinaceous toxin produced by the bacterium *Clostridium botulinum* and related species
- Why are tests needed to detect and quantify BoNT?
  - BoNT is the causative agent in approximately 150 cases/yr of food-borne botulism in the U.S.
  - BoNT was approved by FDA in 1989 for treatment of blepharospasm and strabismus in humans; other conditions have been added
  - In 2008, there were an estimated 5 million off-label cosmetic treatments using BoNT in the U.S[1].
  - BoNT is a Category A bioterrorism threat

[1]Jaspers et al. (2011). Int. J. Oral Maxillofac. Surg. 40: 127–133

2006 NICEATM-ICCVAM-ECVAM Workshop on Alternative Methods for Botulinum Toxin Testing



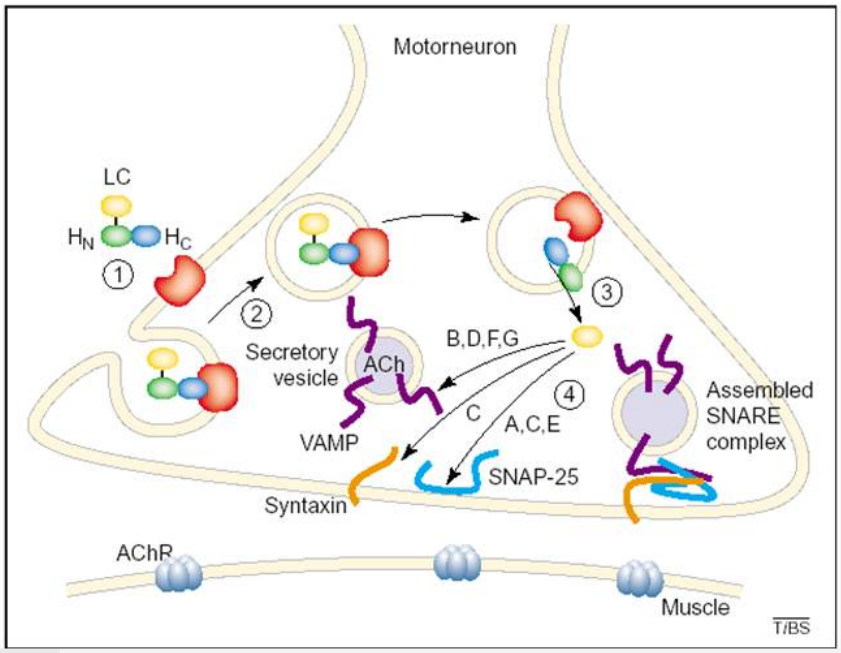
- HSUS nomination
- November 13-14, 2006
  - ICCVAM, NICEATM, ECVAM
  - Silver Spring, MD, USA
- Purpose
  - Review state-of-the-science and current knowledge of 3Rs alternatives
  - Identify priorities for research, development, and validation to advance the use of alternative methods
- >115 registered participants, 10 countries
- Workshop Report:
  - http://iccvam.niehs.nih.gov/methods/biologics/biologics.htm

Replacement Alternatives Evaluated at the Workshop

- Endopeptidase (EP) assay
  - Reflects one but not all of the important modes of toxin action
  - Can provide sensitivity comparable to *in vivo* mouse models
  - Highly specific to toxin serotype
  - May be useful as a replacement if combined with another *in vitro* assay

- Cell-based assay
  - All BoNT biological activities are accounted for
  - Currently, cell-based assays are not a satisfactory replacement
    - Not as sensitive as the mouse bioassay
    - Only work with purified BoNT; cannot test food and environmental samples
    - Different assays do not give similar results with the same cell line
    - No correlation between measured activity and mouse LD_{50} units

Botulinum Toxin Action In Motor Neuron



1. Toxin binds to cell receptor via heavy chain
2. Light chain is internalized
3. Light chain catalyzes the proteolysis of SNARE proteins which are involved in the exocytosis of synaptic vesicles containing acetylcholine
4. Release of acetylcholine at the neuromuscular junction is blocked leading to a flaccid paralysis

Kathryn Turton, John A. Chaddock and K. Ravi Acharya. TRENDS in Biochemical Sciences Vol.27 No.11 November 2002: 552

Workshop Panel Recommendations

- Reviewed methods could be used to reduce or refine the use of mice in the current BoNT test method.
- None could currently be used as a complete replacement for the current BoNT test method.
  - Possible with additional development and validation efforts
- Proposed best practices for BoNT testing include:
  - Use of reference standards to minimize number of replicate animals needed
  - Use of standardized methodology
  - Reduction in the number of doses used in confirmatory testing for potency (e.g., lot release testing)
- Recommended further development and validation efforts for replacement alternatives

Nomination from BioSentinel, Inc.

- Three *In Vitro* Assays - BoTest™, BoTest™ Matrix, BoCell™
- Nominated for consideration for interlaboratory validation studies to evaluate the extent that they can:
  - Detect and quantify Botulinum neurotoxin (BoNT) in a wide range of samples
  - Determine drug product potency
  - Diagnose clinical botulism

Prioritization Criterion 1. Applicable to Regulatory Testing Needs and Agency Programs

- U.S. Regulatory agencies that have needs and/or requirements for the detection, diagnosis, and/or potency testing of BoNTs include:
  - FDA
  - USDA
  - CDC
  - DoD
  - DoI
  - DHS
  - HHS - Biomedical Advanced Research and Development Authority (BARDA)
    - $423M botulism antitoxin contract

Prioritization Criterion 2. Potential to Reduce, Refine, Replace Animal Use

- One mouse potency bioassay can use up to 300 mice - severe pain and distress[a]
- Regulation of products for BoNT-based therapies and cosmetics accounts for an estimated 600,000 animal deaths per year[a]
- BoTest™, BoTest™ Matrix, and BoCell™ intended to be used together for detecting and quantifying BoNT without using animals

[a]Adler et al. 2010

Prioritization Criterion 3. Extent of Expected Use or Application and Impact on Human, Animal, Or Ecological Health

- BoTest™ Matrix applicable to complex samples
- All of the test methods detect and quantify BoNT activity
  - BoTest™ and BoTest™ Matrix Assays detect BoNT proteolytic activity
  - BoCell™ detects BoNT cell binding and uptake, translocation, and proteolytic activities
- Development of new BoNT-based therapies and treatments for botulism is hampered by the lack of high throughput methods to detect and quantify BoNT potency
  - These methods offer the promise of providing such high throughput applications

Prioritization Criterion 4. Potential to Provide Improved Prediction of Adverse Health or Environmental Effects

- BoTest™ and BoTest™ Matrix are useful for the detection of BoNT/A, D, E, F, and G proteolytic activity (only light chain, not full toxin function)
- BoTest™ offers sensitivity near that of the mouse bioassay, depending on the BoNT serotype
  - Offers real-time detection capabilities, tunable sensitivity, and minimal training requirements
- BoCell™ takes into account all of the toxin's cellular activities
  - Offers high throughput assays and minimal equipment investments

Prioritization Criterion 5. Other Advantages

- Do not require extensive instrumentation or specialized training
- Significant cost-savings over traditional animal-based methods

Draft ICCVAM Proposal for Prioritization and Activities

- Should be a high priority for further discussion to determine what is needed to adequately characterize the usefulness and limitations of these *in vitro* assays
  - Will require an assessment of what data are needed and what studies are required to fill any data gaps
  - Studies identified should also be considered high priority

ICCVAM Interagency Biologics Working Group

U.S. Food and Drug Administration

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Questions for SACATM

2. With regard to the nomination of assays developed as alternatives to the mouse assay to detect and/or quantify botulinum neurotoxins (BoNTs) (BioSentinel's *in vitro* BoTest™, the BoTest™ Matrix assays, and the cell-based assay BoCell™) please comment on the proposed ICCVAM priority and activities. Do you agree with the proposed priority and activities, or if not, please explain?